DETAILED ACTION

This action is in response to the amendment, filed 6/18/2009, in which claims 1, 6, 7, 10 and 28 were amended, and claim 30 was newly added. Claims 1, 3-10, 12, 13, 22, 23 and 27-30 are pending.

Applicant's arguments have been thoroughly reviewed, but are not persuasive for the reasons that follow. Any rejections and objections not reiterated in this action have been withdrawn. **This action is FINAL.**

Election/Restrictions

Applicant elected Group I without traverse in the reply filed on 12/5/2005. Currently, claims 1, 3-10, 12, 13, 22, 23 and 27-30 are under consideration.

Priority

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 120 as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35 U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosures of the prior-filed applications, International Application No. PCT/US01/25507 and Provisional Application Nos. 60/225,164 and 60/271,632, fail to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. The prior-filed application numbers do not provide literal or inherent support for the claimed method steps of claims 1, 3-10, 12, 13, 22, 23 and 27-29. While the prior-filed applications suggest that the disclosed method of homologous recombination may be used to construct complex conditional targeting vectors, the specifications do not set forth the claimed method steps. For example, the prior-filed applications do not provide adequate written description for the method step of using homologous recombination to insert a nucleic acid encoding a selectable marker flanked by a pair of second recombining sites and a first recombining site into a second site into the gene in a bacterial artificial chromosome. The prior-filed applications do not teach how to use the disclosed recombination system to make a vector for the conditional knockout of a gene, where two first recombining sites remain in a gene and recombination of the two first sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein.

Claims 1, 3-10, 12, 13, 22, 23 and 27-30 have an effective filing date of 2/12/2003.

Response to Arguments - Claim Objections

The objections of claims 6, 10 and 28 have been withdrawn in view of Applicant's amendment to the claims in the reply filed 6/18/2009.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3-10, 12, 13, 22, 23, 27 and 28 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference) in view of Economides et al (US Patent No. 6,586,251 B2, cited in a prior action; see the entire reference) and Meyers et al (Nature Genetics, Vol. 18, pages 136-141, February 1998, cited in a prior action; see the entire reference). This rejection was made in the Office action mailed 2/19/2009 and is reiterated below.

Lee et al teach an *E. coli*-based system for bacterial artificial chromosome (BAC) engineering (e.g., Title; page 57, left column, 1st full paragraph). Lee et al teach the use of *E. coli* containing a defective λ prophage comprising *gam*, *exo* and *bet* (encoding Gam, Exo and Beta, respectively) under the control of the P_L promoter, which is repressed by the temperature-sensitive repressor CI857 at 32 °C and derepressed at 42 °C, and containing either cre or flpe under the control of an inducible promoter (e.g., Figure 1). Lee et al teach that the λ prophage system appears to be 50-100 fold more efficient than the plasmid based λ recombination system or the RecET system (e.g., page 56, right column, 1st paragraph). Lee et al teach that the recombination system facilitates the generation of complicated conditional targeting vectors (e.g., page 64, right column, 2nd paragraph). Lee et al teach the introduction of a selectable marker flanked by LoxP or FRT sites into an intron of a gene using the bacterial homologous

recombination system, and removal of the selectable marker by inducing transient expression of Cre or Flpe recombinase, respectively (e.g., page 64, right column, 2nd paragraph). Lee et al exemplify a recombination cassette comprising a kanamycin resistance gene flanked by FRT sites (FRT-kan-FRT; e.g., page 57, left column, last full paragraph). Lee et al teach that the bacterial homologous recombination system and reversible expression of Cre or Flpe recombinase greatly speeds up the process of making conditional targeting vectors (e.g., page 64, right column, 2nd paragraph).

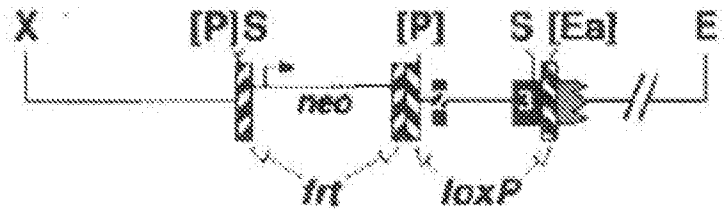
Lee et al do not explicitly teach using homologous recombination to insert a nucleic acid sequence encoding a second selectable marker flanked by a pair of second recombining sites into a second site in the gene; and excising the nucleic acid encoding the second selectable marker with a second recombinase for the second recombining sites, where recombination of the remaining recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein.

Economides et al teach a method for generating large DNA targeting vectors for eukaryotic cells, termed LTVECs (e.g., Abstract; column 3, lines 12-55). Economides et al specifically teach generating an LTVEC for making a conditional allele of a gene (e.g., column 3, line 52; column 10, lines 32-39). Economides et al teach that the problem of engineering precise modifications into very large genomic fragments, such as those cloned into BAC libraries, has largely been solved through the use of homologous recombination in bacteria (e.g., column 2, lines 42-52). Economides et al specifically teach using bacterial homologous recombination to engineer a desired modification within a large genomic fragment, such as a BAC, thereby crating the LTVEC (e.g., column 3, lines 12-22; column 7, lines 53-59; column 8,

lines 16-28). To construct a LTVEC, Economides et al teach (i) obtaining a large genomic clone containing the gene(s) or chromosomal locus (loci) of interest, where the clone is a BAC; and (ii) appending homology boxes to a modification cassette and using bacterial homologous recombination to generate the LTVEC (e.g., column 9, line 12 to column 10, line 14). For LTVEC to make conditional alleles, Economides et al teach the introduction of LoxP sites flanking the region to be excised by Cre recombinase, or FRT sites flanking the region to be excised by Flp recombinase (e.g., column 10, lines 32-39).

Meyers et al teach that the repertoire of allele types that can be generated in mice has been expanded by the development of methods for making gene alteration in mice conditional upon recombination mediated by a site-specific DNA recombinase such as Cre or Flp (e.g., page 136, left column, 2nd paragraph). Meyers et al teach that recombinase-modifiable alleles contain LoxP or FRT sites, the recognition sequences for Cre or Flp, respectively; in the progeny of a cross between mice carrying a conditional allele and transgenic mice carrying the appropriate recombinase gene, recombination between two directly repeated LoxP or FRT sites resolves them to a single site, thereby deleting the gene sequence that lie between them (e.g., page 136, left column, 2nd paragraph). When the targeted locus contains two LoxP sites and two FRT sites, it is modifiable by a Cre, Flp or both (e.g., page 136, left column, 3rd paragraph). Meyers et al teach a targeting vector to produce an allelic series for the mouse *Fgf8* gene. The vector contains *Fgf8* genomic DNA where one LoxP site was inserted in the intron upstream of exon 2, a second LoxP site was inserted in the 3'-untranslated region (UTR) in order to "flox" (flank with LoxP sites) the *Fgf8* coding sequence in exons 2 and 3; and a neomycin-resistance expression cassette flanked by FRT sites (flrted) was inserted immediately upstream of the 5' LoxP site

(e.g., page 136, right column, 1st full paragraph; Figure 1a). Meyers et al teach that the LOXP sites and flrted neo cassette, comprising the promoter and 3'-UTR of the mouse Pgk1 gene, interrupts the *Fgf8* coding sequence (e.g., page 136, right column, 1st full paragraph). The targeting vector is shown in Figure 1a, which is reproduced below (but with the neo gene deleted as a result of recombination between the two Frt sites):



Thus, the two LoxP sites present in the vector flank the region to be conditionally deleted to form a nucleic acid sequence that cannot be transcribed to produce a functional Fgf8 protein (e.g., Figures 1d and 1f).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Lee et al to include the second LoxP or FRT site taught by Economides et al such that the sites flank exons to be deleted as taught by Meyers et al because Lee et al, Economides et al, and Meyers et al teach it is within the ordinary skill in the art to use LoxP and/or FRT sites in the construction of conditional targeting vectors. Lee et al specifically suggest the use of bacterial homologous recombination to insert a nucleic acid sequence encoding a first selectable marker and flanked by a first recombining sites into a first site in the intron of a gene, followed by excising the nucleic acid encoding the selectable marker with a first recombinase specific for the first recombining sites. These steps result in the placement of a first LoxP site in the intron of a gene in a BAC. Economides et al teach the use of BAC vectors to

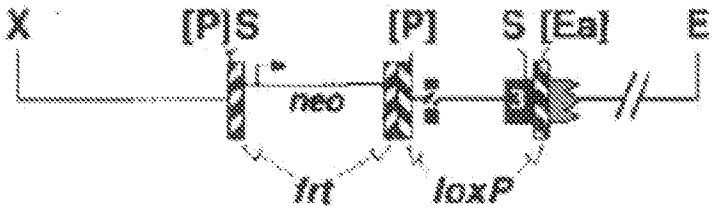
construct conditional allele targeting vectors, where the site-specific recombination sites (LoxP or FRT) flank the region to be excised by the recombinase. Myers et al teach the use of site specific recombination sites to flank exons, which will result in the conditional deletion of a nucleic acid sequence such that a functional protein cannot be produced. Thus, the art teaches where to place the LoxP or FRT sites in the gene targeting vector, and to use BACs modified by bacterial homologous recombination as conditional targeting vectors. To generate a conditional targeting vector suggested by Lee et al, it would have been obvious to one of skill in the art to include the second LoxP or FRT site flanking an exon by repeating the steps of inserting a selectable marker flanked by recombining sites into a second site in the gene, followed by excising the nucleic acid encoding the selectable marker with a recombinase. Since the references teach the use of LoxP or FRT sites, and the sites serve the same function, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have LoxP as the first and second recombining sites, or FRT as the first and second recombining sites. Because both Lee et al and Economides et al teach the use of a kanamycin resistance gene, it would have been obvious to one of ordinary skill in the art to replace the kanamycin resistance gene of Lee et al with the PGK-EM7-neomycin resistance gene cassette, which also provides kanamycin resistance in bacterial cells, in order to achieve the predictable result of providing a recombination cassette for bacterial homologous recombination that can be positively selected by kanamycin resistance.

One would have been motivated to make such a modification in order to receive the expected benefit of providing a vector for conditional targeting as taught by Economides et al with the arrangement of recombination sites taught by Meyers et al using a more efficient

bacterial homologous recombination system for BAC modification as taught by Lee et al. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent any evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Claim 28 requires the steps of (i) using homologous recombination in a bacterial cell to insert a nucleic acid encoding a first selectable marker operably linked to a PGK-EM7 promoter flanked by a pair of LoxP sites into a first site in a gene in a bacterial artificial chromosome, wherein a vector comprises the bacterial artificial chromosome, and wherein the bacterial cell comprises a de-repressible promoter operably linked to a nucleic acid encoding Beta and Exo, and wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta and Exo; (ii) excising the nucleic acid encoding the selectable marker with Cre recombinase, resulting in a single LoxP site remaining in the bacterial artificial chromosome; (iii) using homologous recombination in the bacterial cell to insert a nucleic acid molecule comprising a FRT site 3' and 5' of a nucleic acid encoding a second selectable marker operably linked to PGK-EM7 promoter, wherein the nucleic acid molecule further comprises a LoxP site 3' of the 3' FRT site; and (iv) excising the nucleic acid encoding the second selectable marker with FLP, resulting in a second LoxP site and a FRT site remaining in the bacterial artificial chromosome, thereby generating the vector for conditional knockout of a gene. Lee et al teach the step of inserting a nucleic acid encoding a selectable marker flanked by LoxP sites into a gene (first site) in a bacterial artificial chromosome using bacterial homologous recombination, where the bacterial cell for recombination comprises a de-repressible promoter operably linked to a nucleic acid encoding Beta and Exo, and where using

homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta and Exo. Further, Lee et al teach excising the selectable marker by transiently expressing Cre recombinase. Lee et al do not specifically teach the use of a PGK-EM7 promoter flanked by a pair of LoxP sites. However, both Lee et al and Economides et al teach the use of a kanamycin resistance marker flanked by site-specific recombination sites, and Economides et al teach the use of the PGK-EM7 promoter. Thus, it would have been obvious to one of ordinary skill in the art at the time the invention was made to include the PGK-EM7 promoter to drive the expression of the selectable marker in order to achieve the predictable result of expressing the selectable marker in the bacteria in which homologous recombination occurs. Further, Lee et al do not teach using homologous recombination in the bacterial cell to insert a nucleic acid molecule comprising a FRT site 3' and 5' of a nucleic acid encoding a second selectable marker operably linked to PGK-EM7 promoter, wherein the nucleic acid molecule further comprises a LoxP site 3' of the 3' FRT site; and excising the nucleic acid encoding the second selectable marker with FLP, resulting in a second LoxP site and a FRT site remaining in the bacterial artificial chromosome, thereby generating the vector for conditional knockout of a gene. However, Meyers et al teach a targeting vector of the following structure:



This vector has a single LoxP site inserted into the 3'UTR of the gene. Further, it contains a neomycin reporter flanked by FRT sites and further comprising a LoxP site 3' of the 3' FRT site.

It would have been obvious to one of ordinary skill in the art to make this structure using the bacterial recombination system of Lee et al, where the neo cassette comprises the PGK-EM7-neo cassette of Economides et al, because both Meyers et al and Economides et al teach the use of a neomycin cassette, and Lee et al teaches the use of a kanamycin cassette. The neomycin resistance gene provides kanamycin resistance in bacterial cells. Thus, one would use the PGK-EM7-neo cassette in order to achieve the predictable result of providing a kanamycin resistance cassette capable of being expressed in the bacteria used for homologous recombination. It would have been obvious to one of ordinary skill in the art to insert the FRT-neo-FRT-LoxP sequence shown in the targeting vector of Meyers et al as a single recombination event using the bacterial recombination system of Lee et al, because Lee et al teach the system for inserting a selectable marker flanked by recombination sites, and the LoxP site of Meyers et al is immediately 3' to the FRT site. One would recognize that this portion of the construct could be inserted in a single step to save time. Furthermore, Meyers et al teach that the neomycin resistance gene can be removed by FLP recombinase prior to the production of a mouse line in order to prevent the creation of a hypomorphic allele (e.g., paragraph bridging pages 136-137). It would have been obvious to one of skill in the art to remove the neomycin resistance gene in order to receive the expected benefit of not affecting the level of transcript prior to the deletion of the portion of the gene flanked by LoxP sites in the targeted mouse.

Claims 29 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lee et al (Genomics, Vol. 73, pages 56-65, 2001, cited in a prior action; see the entire reference) in view of Economides et al (US Patent No. 6,586,251 B2; see the entire reference) and Meyers et

al (Nature Genetics, Vol. 18, pages 136-141, February 1998; see the entire reference) as applied to claims 1, 3-10, 12, 13, 22, 23 and 27 above, and further in view of Stewart et al (US Patent No. 6,355,412 B1, cited in a prior action; see the entire reference). This rejection was made over claim 29 in the Office action mailed 2/19/2009 and has been extended to new claim 30.

The combined teachings of Lee et al, Economides et al and Meyers et al are described above and applied as before.

Lee et al, Economides et al and Meyers et al do not teach the length of the sequence homologous to the site in the bacterial artificial chromosome.

Stewart et al teach a method of performing homologous recombination in a host cell, comprising introducing a nucleic acid sequence encoding RecE/T or Redα/β recombinase (i.e. Lambda Exo and Beta) into a host cell, introducing a polynucleotide comprising a nucleotide sequence homologous to the nucleotide sequence of interest into the host cell, activating the expression of RecE/T, and selecting a cell from the population in which homologous recombination has occurred (e.g. column 28, lines 10-50; column 29, lines 9-35; column 28, line 51 to column 29, line 8; columns 25-27; paragraph bridging columns 37-38). Further, Stewart et al teach the use of Gam in addition to Exo and Beta or RecE/T (e.g. column 25, lines 5-28; Example 1). Stewart et al teach that a variety of host-vector systems may be utilized to introduce and express the protein-coding sequence of RecE/T or Redα/β, including prokaryotic and eukaryotic cells such as bacterial, yeast, plant, rodent, mice, human, insect or mammalian cells (e.g. column 28, lines 10-40). With respect to regulatory controls, Stewart et al teach that a range of different expression levels and a variety of regulatory sequences are known in the art and the ability to generate a wide range of expression is advantageous for utilizing the method

(e.g. column 25, lines 5-44; column 24, line 50 to column 25, line 3). Stewart et al teach that the expression can be regulated by the P_L promoter of phage λ and the inducible lambda repressor CI_{857} (e.g. column 26, lines 1-27). Stewart et al teach that the nucleotide sequence of interest may be extrachromosomal and located on a bacterial artificial chromosome (e.g. column 20, lines 37-57; paragraph bridging columns 28-29). Stewart et al teach that homology arms are required for recombination and are two short regions of double-stranded DNA homologous to the sequence of the target DNA of interest (e.g., paragraph bridging columns 19-20). Stewart et al teach that the homology arms contain approximately 22 to 100 base pairs or more of continuous identity to a double-stranded region flanking the DNA of interest, which results in 44-200 bp of sequence homologous to the target site (e.g., paragraph bridging columns 19-20). Stewart et al teach that the lambda recombinases can be used to achieve high-efficiency targeted cloning (e.g. column 11, lines 3-47). Moreover, Stewart et al teach that when the recombination method is used in combination with site-specific recombination sites, the site-specific recombinase, which recognizes the sites is under the control of an inducible promoter such that upon induction of recombination expression, recombination between the site-specific recombination sites occurs (e.g., Figure 4).

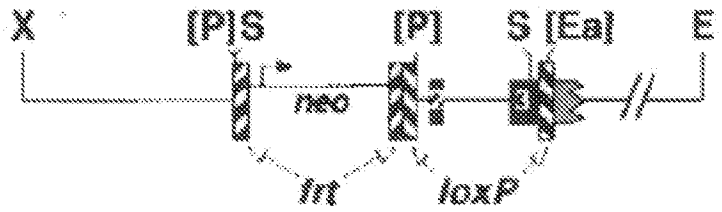
Because Lee et al and Stewart et al teach the use of bacterial homologous recombination mediated by λ Beta, Exo and Gam, and Stewart et al teach the length of homology required for bacterial recombination mediated by λ Beta, Exo and Gam, it would have been obvious to one of ordinary skill in the art at the time the invention was made to use regions of homology of 22-100 bp on each end of the sequence to be inserted in order to achieve the predictable result of providing regions of homology sufficient to promote bacterial homologous recombination.

Response to Arguments - 35 USC § 103

With respect to the rejection of claims 1, 3-10, 12, 13, 22, 23, 27 and 28 under 35 U.S.C. 103(a) as being unpatentable over Lee et al in view of Economides et al and Meyers et al, Applicant's arguments filed 6/19/2009 have been fully considered but they are not persuasive.

At page 6, the response asserts that the constructs disclosed in the cited prior art could not achieve the desired effect if two different recombinases were used with the disclosed constructs.

This argument is not found persuasive. Meyers et al teach a targeting vector of the following structure:



This vector has a single LoxP site inserted into the 3'UTR of the gene. Further, it contains a neomycin reporter flanked by FRT sites and further comprising a LoxP site 3' of the 3' FRT site. Meyers et al teach that the neomycin resistance gene can be removed by FLP recombinase prior to the production of a mouse line in order to prevent the creation of a hypomorphic allele (e.g., paragraph bridging pages 136-137). If the 3' LoxP site is inserted using the method of Lee et al (i.e., insertion of a selectable marker flanked by LoxP sites followed by recombination of the two LoxP sites with Cre recombinase), a single 3' LoxP site remains in the vector. If the second recombination event uses a substrate comprising a selectable marker flanked by frt sites and further comprising a 3' LoxP site, recombination of the frt sites with flpe

recombinase results in a single frt site, a 5' LoxP site and a 3' LoxP site, wherein recombination between the two remaining LoxP sites would result in a sequence that cannot be transcribed to produce a functional protein. Thus, the statement that two different recombinases cannot be used is not accurate.

At pages 6-7, the response asserts that Lee et al do not suggest, or render obvious inserting a second nucleic acid encoding a selectable marker flanked by a pair of second recombining sites into a second site in the gene. In addition, the response asserts that Lee et al do not suggest, nor render obvious, excising the nucleic acid encoding the selectable marker with a second recombinase specific for the second recombining sites, wherein two first recombining sites remain in the gene following excision of the nucleic acid encoding the selectable marker, and wherein recombination of the first two recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein. Further, the response points to the previously filed declaration of E-Chiang Lee and Pentao Liu under 37 CFR 1.132 as delineating the differences between Lee et al and the presently claimed methods. The response asserts that the teachings of Lee et al do not provide any information on how to produce a nucleic acid sequence that cannot be transcribed into a functional protein. The response points to the portions of Lee et al that are directed to the expression of Cre protein from an untranslated region of the *Eno2* gene.

These arguments are not found persuasive. Lee et al specifically suggest the use of the disclosed recombination system for the construction of conditional targeting vectors. Page 64, right column, paragraph 2 is reproduced here:

> This recombination system also facilitates the generation of complicated conditional targeting vectors. While the generation of such vectors often used to take several months, it can now be performed in a only few weeks. The ability to express reversibly Cre or Flpe recombinases in *E. coli* speeds this process even further. A selectable marker flanked with *lox*P or *FRT* sites can now be introduced into an intron of a gene and then removed by transient Cre or Flpe expression, leaving behind a solo *lox*P or *FRT* site in the intron. A limitation of this approach at the present time is the lack of a BAC-based mouse physical map and the paucity of mouse genome sequence information. This should all dramatically change, however, next year, as the draft sequence of the mouse comes on-line and the BAC physical map is completed.

Although Lee et al only mentions the introduction of a single LoxP or FRT site into the intron, one of skill in the art would have immediately recognized that a conditional targeting vector would require two LoxP or two FRT sites flanking a portion of a gene in order to be an effective conditional targeting vector. The requirement for two site-specific recombination sites is evidenced by the teachings of Economides et al and Meyers et al. It would have been obvious to one of ordinary skill in the art to apply the homologous recombination technique taught by Lee et al to construct a conditional targeting vector, because Lee et al specifically suggest this application of their method. Further, it would have been obvious to apply the technique of Lee et al to the introduction of two site-specific recombination sites flanking a region of a gene to generate a conditional targeting vector, because the art teaches that two sites are needed in order to make a conditional targeting vector. One would have made such a modification to improve

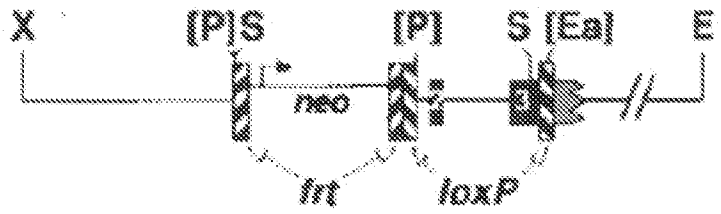
the efficiency of the process of making the targeting vector, as taught by Lee et al. In other words, Lee et al teach the application of the process to the construction of conditional targeting vectors, and the art teaches that a second site is mandatory for conditional targeting vectors. It is implicit that the conditional targeting vector of Lee et al will have a second site, and this requirement is made explicit by Economides et al and Myers et al.

At page 7, the response notes that Economides et al describe methods for using large DNA vectors to target, via homologous recombination, chromosomal loci in eukaryotic cells. The large DNA targeting vectors are termed LTVECs and can be used for the creation of conditional alleles. The response notes that Economides et al state that a conditional allele can be generated "by introduction of LoxP sites flanking the region to be excised by Cre recombinase [citation deleted] or FRT sites flanking the region to be excised by Flp recombinase." Thus, the response assert that Economides et al teach that a single pair of recombining sites can be used to produce a LTVEC that can conditionally "knock-out" a section of an LTVEC. The response asserts that Economides do not teach the use of a second pair of recombining sites flanking a selectable marker, nor do they teach any specific steps in a method for producing the construct. At pages 8-9, the response discusses the teachings of Myers et al. The response asserts that it is important to note that the teachings of Myers et al are designed to determine the effect of altered splicing of the *Fgf8* gene through the inclusion of the neo cassette.

The structure taught by Economides et al is the structure that results from the method of instant claim 1, for example. The conditional targeting vector that is the product of claim 1 comprises a single pair of recombining sites that when recombined will produce a sequence that cannot be transcribed or translated to produce a functional protein.

Meyers et al teach that the **neomycin resistance gene can be removed by FLP recombinase prior to the production of a mouse line** in order to prevent the creation of a hypomorphic allele (e.g., paragraph bridging pages 136-137). The conditional allele of Myers et al does not contain a neomycin gene (e.g., Figure 1e). Thus, Myers et al teach a conditional targeting vector that comprises a single Frt site followed by a 3' LoxP site and a second LoxP site, where the two LoxP sites flank a portion of the gene to be deleted. This structure falls within the scope of the resulting structures of claim 1 and specifically results from the method of claim 28.

Given the combined teachings of Lee et al, Economides et al, and Myers et al, one of ordinary skill in the art at the time the invention was made could have readily envisioned the application of the method of Lee et al to the construction of a conditional targeting vector as shown below **but with the deletion of the neo gene as a result of recombination between the two Frt sites**:



The method of Lee et al is based upon the insertion of a selectable marker flanked by site-specific recombination sites followed by the expression of the site-specific recombinase. Expression of the site-specific recombinase results in the recombination of the two flanking recombination sites and removal of the selectable marker, leaving a single recombination site. Thus, to insert the most 3' LoxP site of the vector, one would have recognized that one could use

the method of homologous recombination of Lee et al to insert a nucleic acid encoding a first selectable marker flanked by a pair of first LoxP recombining sites into a first 3' site in the gene in a bacterial artificial chromosome, followed by excision of the selectable marker with Cre recombinase to leave a single 3' LoxP recombining site. To insert the 5' Frt and LoxP sites, one would have recognized that one could use the homologous recombination method of Lee et al to insert a nucleic acid comprising a second selectable marker flanked by a pair of Frt sites and a single 3' LoxP site, followed by excision of the selectable marker with Flpe recombinase to leave a single 5' Frt site and a single 5' LoxP site. The resulting **single pair** of **LoxP** sites are positioned such that recombination between these two sites result in a nucleic acid sequence that cannot be transcribed to produce a functional protein. The vector is a conditional targeting vector, because the gene can be transcribed to produce a functional protein until recombination between the single pair of LoxP sites is performed. The knock-out of the gene would be conditional upon the recombination of the single pair of LoxP sites remaining in the vector.

At page 9, the response asserts that Myers et al teach away from the claimed invention because if two copies of a neo gene were included in the construct, the construct would be completely ineffective to achieve the desired result.

This argument is not found persuasive, because Myers et al teach the removal of the neo gene in order to make a conditional knock-out allele. Thus, the rejection of record is based upon the complete absence of a neo allele in the conditional targeting vector.

At page 9, the response asserts that even if one combined the teachings of the cited references, one would not arrive at the claimed methods. The response asserts that one would produce only constructs with a single pair of recombining sites flanking a selectable marker.

This argument is not found persuasive. Lee et al teach the use of homologous recombination to insert a selectable marker flanked by a pair of recombining sites, followed by expression of a site-specific recombinase to remove the selectable marker, thereby resulting in a single site-specific recombination site (e.g., LoxP). Lee et al teach the application of this method to make a conditional targeting vector. Economides et al and Myers et al teach that a pair of site-specific recombination sites must flank a portion of a gene in order to make a conditional targeting vector. Thus, one would have recognized the need to insert a second site-specific recombination site. Examination of the structure taught by Myers et al would have led one of skill in the art to use a second step of homologous recombination to insert a selectable marker flanked by a second pair of recombining sites (different than the first, e.g., Frt) and an additional first recombining site (LoxP), followed by expression of a site-specific recombinase to recombine the second pair of recombining sites, leaving a pair of first sites (e.g., LoxP).

At page 9, the response asserts that Lee et al, Economides et al and Myers et al, even in combination, do not suggest: (1) the use of two selectable markers, each flanked by a pair of recombining sites; or (2) any specific steps in any method that would lead to a vector for a conditional knockout where two selectable markers are utilized.

This argument is not persuasive. Lee et al teach the use of homologous recombination to insert a selectable marker flanked by a pair of recombining sites, followed by expression of a site-specific recombinase to remove the selectable marker, thereby resulting in a single site-specific recombination site (e.g., LoxP). Lee et al teach the application of this method to make a conditional targeting vector. Economides et al and Myers et al teach that a pair of site-specific recombination sites must flank a portion of a gene in order to make a conditional targeting

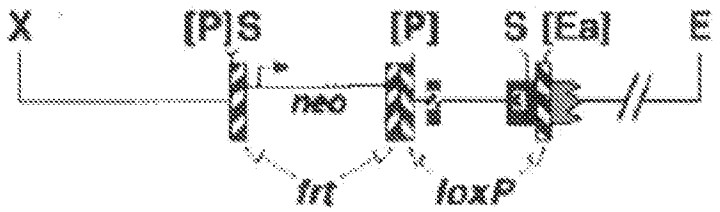
vector. Thus, one would have recognized the need to insert a second site-specific recombination site. To insert the second site, one would have applied the steps of the method of Lee et al. Economides et al specifically suggest the use of a bacterial homologous recombination method to make the conditional targeting vector, and the method taught by Lee et al is a bacterial homologous recombination method. Accordingly, one would have been applying a known technique to improve the construction of conditional targeting vectors to yield predictable results.

At the paragraph bridging pages 9-10, the response asserts that Myers et al teach that there are substantial effects caused by inclusion of a selectable marker in the construct.

This argument is not found persuasive. The teachings of Myers et al that are directed to the creation of hypomorphic alleles by inclusion of a selectable marker are not relied upon in the rejections of record. Myers et al teach the removal of the selectable marker to make a conditional allele. The combined teachings of Lee et al, Economides et al, and Myers et al result in a conditional targeting vector that does not contain a selectable marker in the gene.

At page 10, the response asserts that it is not clear how the references can be combined to result in the method of claim 28.

Given the combined teachings of Lee et al, Economides et al, and Myers et al, one of ordinary skill in the art at the time the invention was made could have readily envisioned the application of the method of Lee et al to the construction of a conditional targeting vector as shown below **but with the deletion of the neo gene as a result of recombination between the two Frt sites**:



The method of Lee et al is based upon the insertion of a selectable marker flanked by site-specific recombination sites followed by the expression of the site-specific recombinase. Expression of the site-specific recombinase results in the recombination of the two flanking recombination sites and removal of the selectable marker, leaving a single recombination site. Thus, to insert the most 3' LoxP site of the vector, one would have recognized that one could use the method of homologous recombination of Lee et al to insert a nucleic acid encoding a first selectable marker flanked by a pair of first LoxP recombining sites into a first 3' site in the gene in a bacterial artificial chromosome, followed by excision of the selectable marker with Cre recombinase to leave a single 3' LoxP recombining site. To insert the 5' Frt and LoxP sites, one would have recognized that one could use the homologous recombination method of Lee et al to insert a nucleic acid comprising a second selectable marker flanked by a pair of Frt sites and a single 3' LoxP site, followed by excision of the selectable marker with Flpe recombinase to leave a single 5' Frt site and a single 5' LoxP site. The resulting **single pair** of **LoxP** sites are positioned such that recombination between these two sites result in a nucleic acid sequence that cannot be transcribed to produce a functional protein. The vector is a conditional targeting vector, because the gene can be transcribed to produce a functional protein until recombination between the single pair of LoxP sites is performed. The knock-out of the gene is conditional upon the recombination of the single pair of LoxP sites remaining in the vector.

The response asserts that a large number of experiments were required to devise the presently claimed methods. The response asserts that the Examiner has not considered the additional information in the declaration of Dr. Lee. The response asserts that Dr. Lee was not able to simply use the work he presented in Lee et al to quickly devise methods for producing vectors for conditional knockouts. The response asserts that the large number of required experiments is evidenced in data and methods presented in the declaration. The response asserts that this is sufficient evidence of secondary considerations (difficulty in producing the claimed invention) to overcome the rejections.

These arguments have been fully considered but are not persuasive. It was recognized in the art that the production of conditional targeting vectors is complicated and can take several months (Lee et al, page 64, right column, 2nd paragraph). Lee et al teach that the disclosed homologous recombination system can be applied to the construction of conditional targeting vectors and that the homologous recombination system is highly efficient (e.g., Discussion). Paragraph 3 of the declaration filed 1/12/2008 states, "The recombineering method allows one person to make a conditional knockout vector and several vectors simultaneously in 2-3 weeks." This is completely consistent with the teachings of Lee et al and does not provide evidence of difficulty in making the claimed invention by the present inventors or by others.

At paragraph 3 of the declaration filed 3/7/2008, the declaration notes that a declaration under 37 C.F.R. § 1.131 is of record. These experiments demonstrate the successful application of homologous recombination in bacteria to insert a selectable marker flanked by LoxP sites into a gene followed by the expression of Cre to remove the selectable marker. Difficulty in making

the claimed invention by the present inventors or by failure by others is not evidenced by these experiments.

At paragraph 4 of the declaration filed 3/7/2008, it was states that additional data was provided documenting that the vectors produced using the claimed methods were introduced into ES cells in the United States prior to February 13, 2002. This data is not directed to experiments to devise the presently claimed method. Rather, this data is directed to the use of the product produced by the claimed methods.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

With respect to the rejection of claims 29 and 30 under 35 U.S.C. 103(a) as being unpatentable over Lee et al in view of Economides et al and Meyers et al, and further in view of Stewart et al, Applicant's arguments filed 6/18/2009 have been fully considered but they are not persuasive.

The response asserts that there is no description in Stewart et al to describe (1) the use of two selectable markers, each flanked by a pair of recombining sites; (2) the use of homologous recombination to make vectors for conditional knock-outs; or (3) specific steps in any method that would lead to a vector for a conditional knockout, let alone a method that includes the use of both LoxP and FRT sites. Thus, the response asserts that Stewart et al do not make up for the deficiencies of Lee et al, Economides et al or Myers et al.

This argument is not found persuasive for the reasons set forth above. Stewart et al was cited to teach the length of the homology arms. Applicant agrees that Stewart et al do teach the use of homology arms (page 11, paragraph 3). The asserted deficiencies in Lee et al,

Economides et al and Meyers et al are not deficiencies for the reasons set forth above with regard to the rejection of claims 1, 3-10, 12, 13, 22, 23, 27 and 28.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached at 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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Examiner
Art Unit 1636

/JD/

/ Christopher S. F. Low /
Supervisory Patent Examiner, Art Unit 1636